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Szyf et al.

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[54] DNA METHYLTRANSFERASE ANTISENSE OLIGONUCLEOTIDES

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[21] Appl. No.: 161,673

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[51] Int. Cl.[6] C07H 21/00; A61K 48/00

[52] U.S. Cl. 536/24.5

[58] Field of Search 514/44; 536/24.5

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Assistant Examiner—D. Curtis Hogue, Jr.
Attorney, Agent, or Firm—Hale and Dorr

[57] ABSTRACT

The invention encompasses tumorigenicity-inhibiting antisense oligonucleotide sequences complementary to mRNA or double-stranded DNA that encodes mammalian DNA methyl transferase. It further encompasses methods for inhibiting tumorigenicity and pharmaceutical composition comprises the tumorigenicity-inhibiting antisense nucleotide.

4 Claims, 4 Drawing Sheets

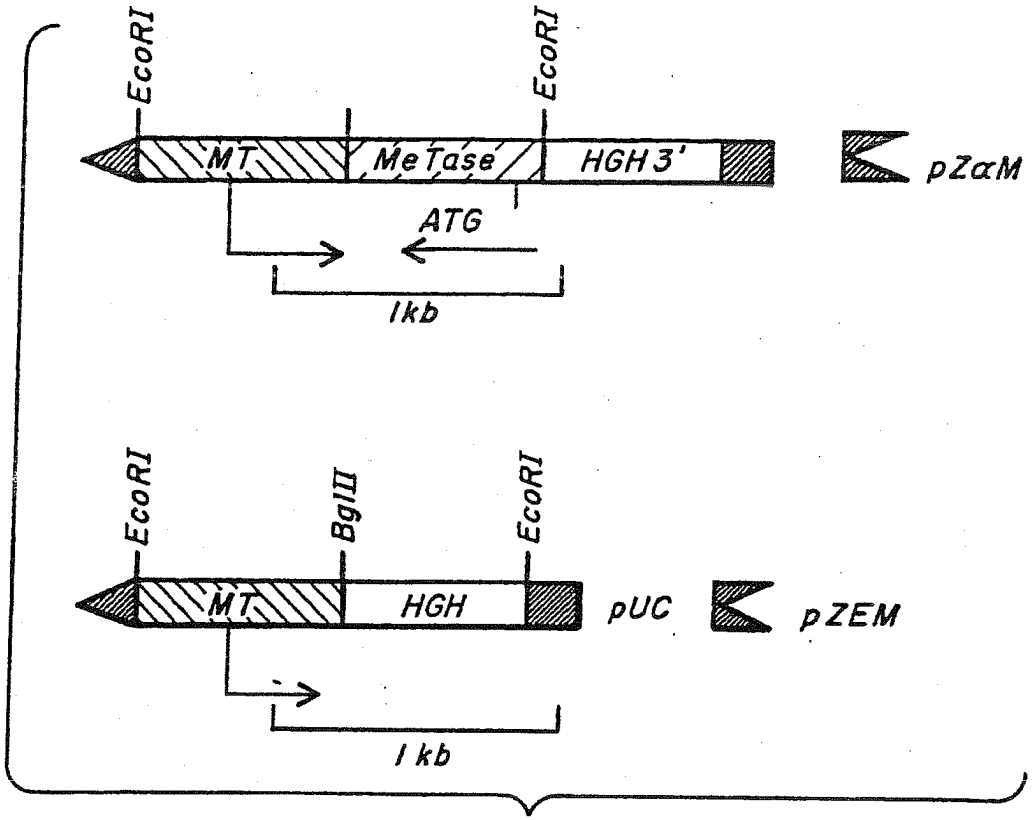

EcoRI
EcoRI
MT
MeTase
HGH 3'
pZαM
ATG
1kb
EcoRI
BglII
EcoRI
MT
HGH
pUC
pZEM
1 kb

FIG. 1

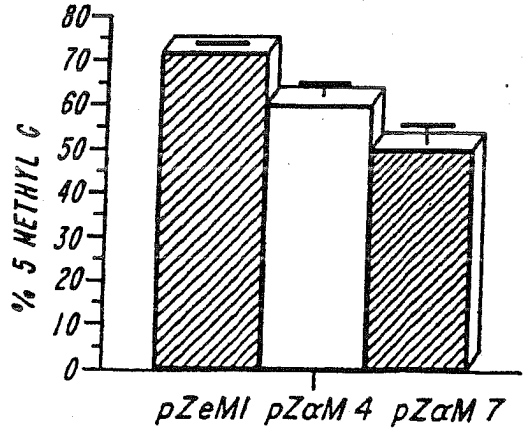

% 5 METHYL C
80
70
60
50
40
30
20
10
0
pZeMI
pZαM 4
pZαM 7

FIG. 2

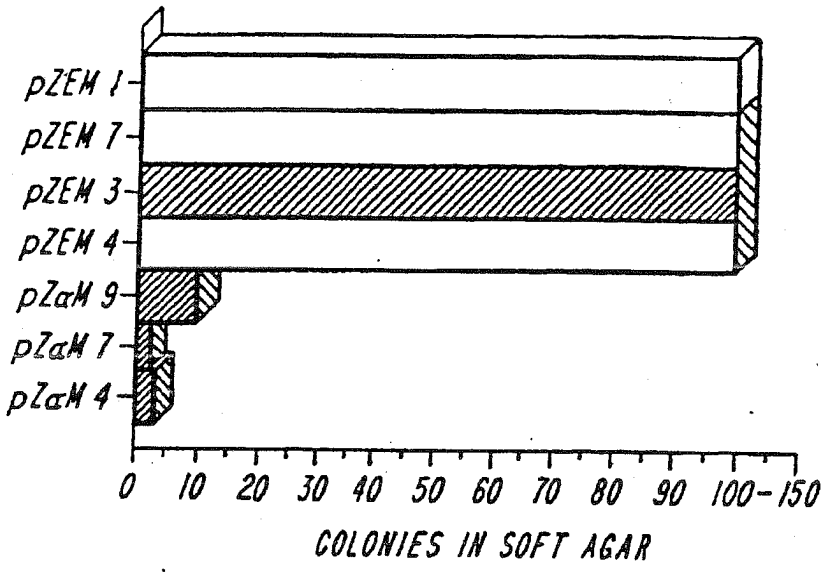

pZEM 1
pZEM 7
pZEM 3
pZEM 4
pZαM 9
pZαM 7
pZαM 4
0 10 20 30 40 50 60 70 80 90 100-150
COLONIES IN SOFT AGAR

FIG. 3

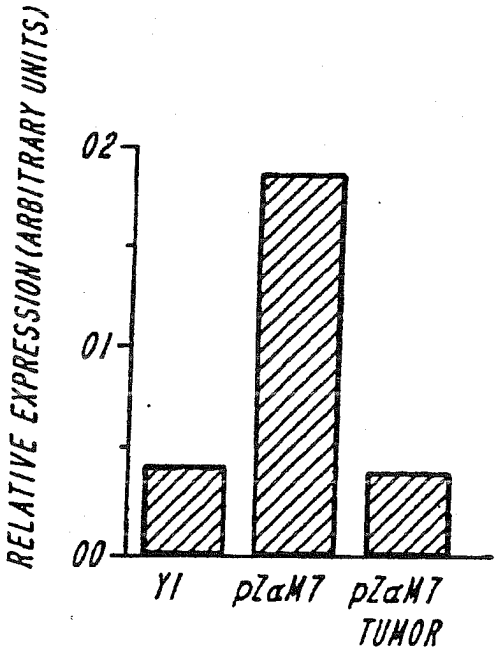

RELATIVE EXPRESSION (ARBITRARY UNITS)
02
01
00
YI
pZαM7
pZαM7
TUMOR

FIG. 4

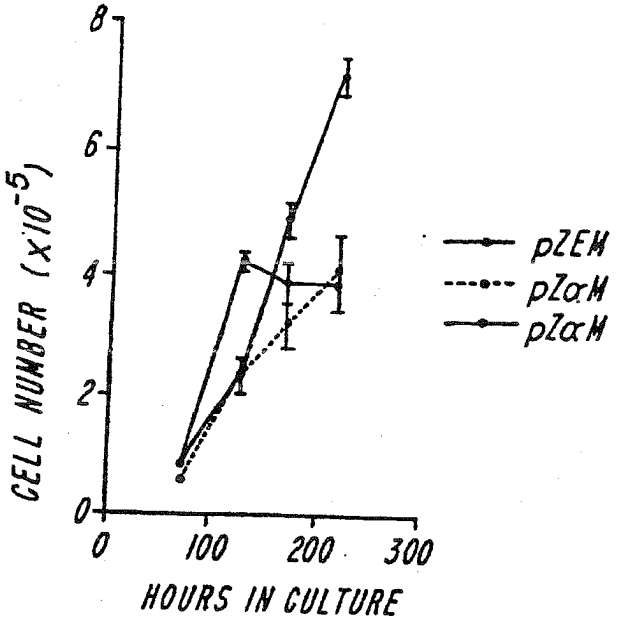

8
6
4
2
0
CELL NUMBER ($\times 10^{-5}$)
0
100
200
300
HOURS IN CULTURE
pZEM
pZαM
pZαM

FIG. 5A

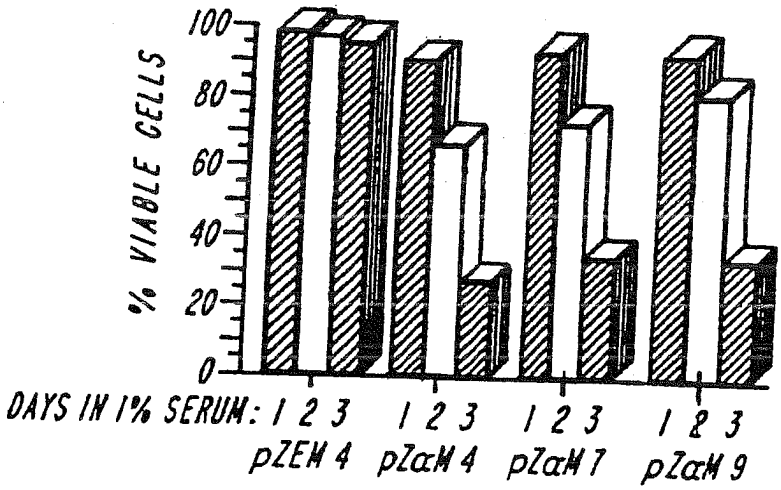

100
80
60
40
20
0
% VIABLE CELLS
DAYS IN 1% SERUM: 1 2 3 1 2 3 1 2 3 1 2 3
pZEM 4 pZαM 4 pZαM 7 pZαM 9

FIG. 5B

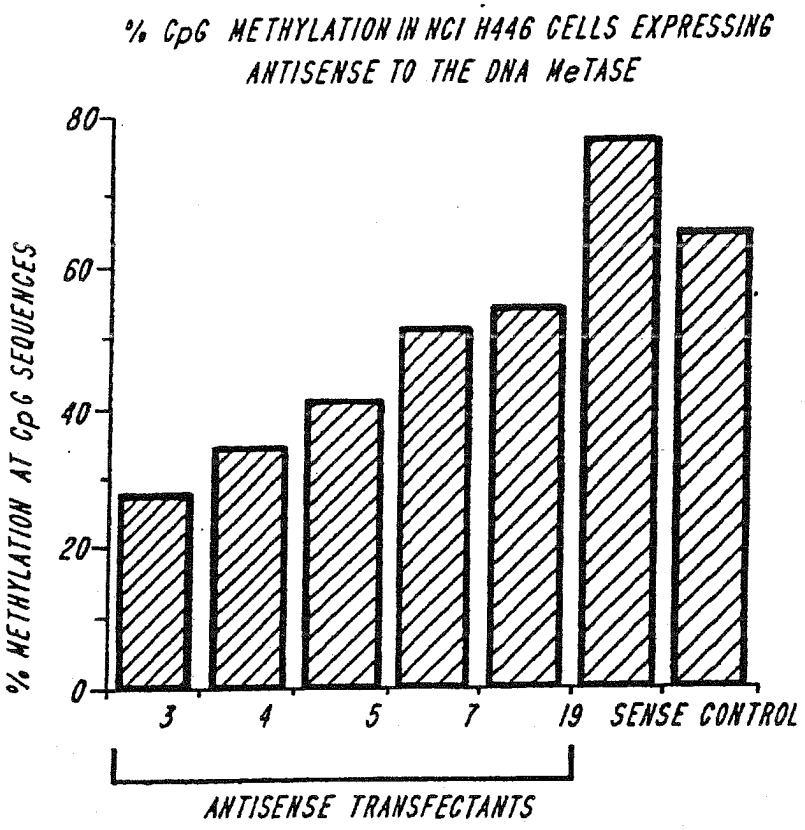

% CpG METHYLATION IN NCI H446 CELLS EXPRESSING ANTISENSE TO THE DNA MeTASE
% METHYLATION AT CpG SEQUENCES
80
60
40
20
0
3
4
5
7
19
SENSE CONTROL
ANTISENSE TRANSFECTANTS

FIG. 6

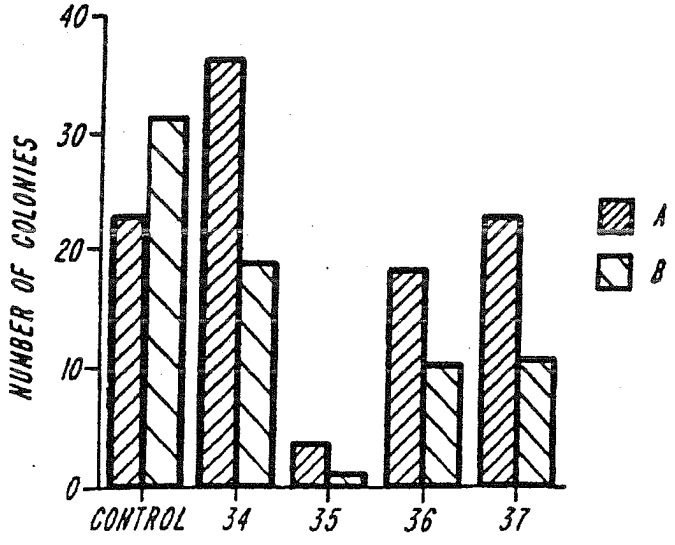

NUMBER OF COLONIES
40
30
20
10
0
CONTROL
34
35
36
37
A
B

FIG. 7

DNA METHYLTRANSFERASE ANTISENSE OLIGONUCLEOTIDES

FIELD OF THE INVENTION

The present invention provides antisense oligonucleotides that surprisingly demonstrate tumorigenicitiy-inhibiting activity. The inventive oligonucleotides inhibit tumorigenisis by inhibiting expression of the gene encoding DNA methyl transferase. These oligonucleotides are complemen-

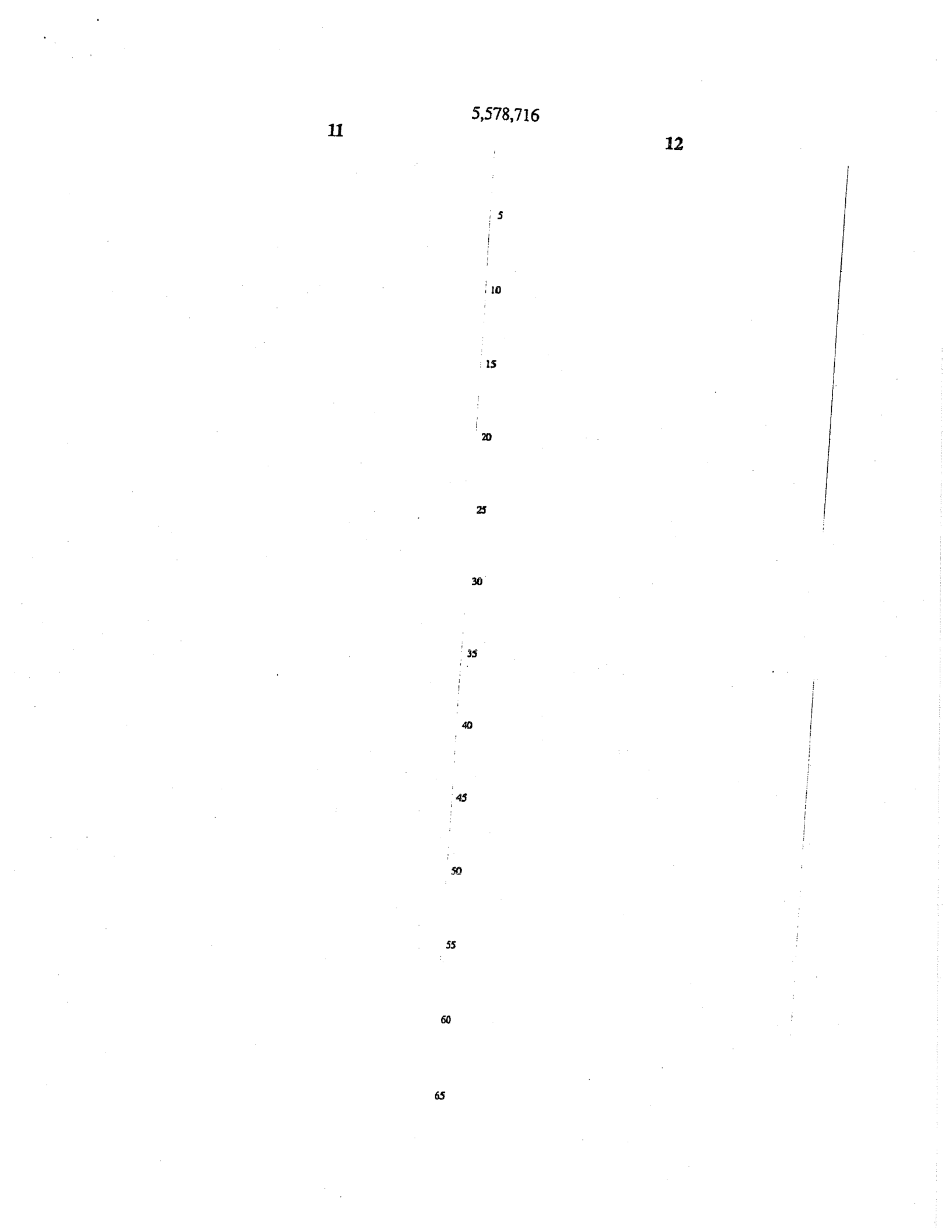
In a fifth embodiment, tumorigenicity-inhibiting modified oligonucleotides are self-stabilized by having a self-complementary region that hybridizes intramolecularly with the oligonucleotide to form an exonuclease resistant hairpin-like structure (see e.g., Agrawal et al., *Nucleic Acids Res.* 20: 2729–2735 (1993). Modified oligonucleotides according to this embodiment of the invention are generally characterized by having two regions: a DNA MeTase hybridizing region and a self-complementary region.

complementary region. The self-complementary region may also contain oligonucleotide sequences that are complementary to the tumorigenicity hybridizing region.

Either the hairpin structure or the hammer-like structure will presumably have loops of 4 or more nucleotides resulting from non-base-paired nucleotides.

In a preferred embodiment, there are about 10 intramolecular base-pairs formed in the self-stabilized oligonucleotide, with the 10 base pairs being consecutive and involving the 3'-most nucleotides. Of course, the intramolecular base-pairing can be so extensive as to involve every nucleotide of the oligonucleotide. Preferably, this will involve a self-complementary region of about 50 nucleotides or less.

To directly inhibit DNA methylation in Y1 cells, either the DNA MeTase antisense expression construct pZαM or a pZEM control vector, Szyf, et al., *J. Biol. Chem.*, 267: 12831–12836 (1992)) was introduced into Y1 adrenocortical carcinoma cells by DNA-mediated gene transfer as follows.


5
10
15
20
25
30
35
40
45
50
55
60
65

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 12

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i v) ANTI-SENSE: YES

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CATCTGCCAT TCCCACTCTA

20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i v) ANTI-SENSE: YES

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTGGCATCTG CCATTCCCAC TCTA

24

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..19
(D) OTHER INFORMATION: /note="5'PRIMER BASES 154-172"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TCCGAATCGG TTTCCACCC

19

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:

-continued

(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /note="3'PRIMER BASES 472-492"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGAGGATGAG GGCCTGAATG C

2 1

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..18
(D) OTHER INFORMATION: /note="PRIMER 1-18"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGACTGGGGT GAGGACGG

1 8

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..24
(D) OTHER INFORMATION: /note="PRIMER 620-610"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TTTCAGTAGA TAACGCACTG CTGG

2 4

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..19
(D) OTHER INFORMATION: /note="ANTI SENSE PRIMER"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCAAACAGAA TAAAGAATC

1 9

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:

-continued

(A) NAME/KEY: misc_feature
(B) LOCATION: 1..19
(D) OTHER INFORMATION: /note="SENSE PRIMER"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTATGGTGGT TTGCCTGGT

19

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..20
(D) OTHER INFORMATION: /note="Oligo 34: DW2-34B (antisense phosphodiester)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CATCTGCCAT TCCCACTCTA

20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..20
(D) OTHER INFORMATION: /note="Oligo 35: DW2-35C (antisense phosphorothioate)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATCTGCCAT TCCCACTCTA

20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..20
(D) OTHER INFORMATION: /note="Oligo 36: DW2-36C (Random Control Phosphodiester)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTGACTGCCA ACTATGAACA

20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs

-continued

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..20
(D) OTHER INFORMATION: /note="Oligo 37: DW2-37D (Random Control Phosphorothioate)"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTGACTGCCA ACTATGAACA

20